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BIOLOGICAL BULLETIN

MANGANESE OF THE LAMELLIBRANCHS.

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In 1892 Griffiths published an account of the finding of manganese in the blood of the lamellibranch, *Pinna squamosa*.[1] So far as I am aware this result has never been confirmed nor until recently has an examination of other molluscs led to an extension of this isolated fact. To the student of comparative physiology such a finding must be of considerable interest, adding one more respiratory mechanism to the list of five or six with which we are familiar. At the same time it is highly improbable that *Pinna squamosa* is the only mollusc utilizing manganese in a respiratory compound; we should expect to find the element in other forms more or less closely related to it. It is a matter of common observation that the respiratory proteins of the more highly organized animals fall into a few general types, such as hæmoglobin or hæmocyanin, and that while individual bloods may show subtle biological differences within one of these groups, there is never any difficulty in determining chemically whether a blood pigment is a hæmocyanin, a hæmoglobin, or some other typical complex. The effective respiratory mechanisms are thus quite limited, so that we do not expect to find a single member of a family possessed of a blood protein totally unlike the other members of that family.

For this reason we have extended the investigation of this point somewhat with a view to determining what other lamellibranchs are provided with a respiratory mechanism similar to that of *Pinna squamosa*. The most notable group which we

[1]Griffiths, *Compt. rend. de l'Acad. des Sci.*, CXIV., p. 840.

have thus far found to utilize the element manganese is the Unionidæ, the common fresh-water mussels. Since 1906 when the element was first noticed in the specimens obtained from the Madison Lakes of Wisconsin,[1] we have examined many hundreds of specimens from the Mississippi basin, St. Lawrence and Atlantic coast drainage. In not a single specimen has the element been wanting or small in amount. It is obvious that a single individual which failed to show manganese, or contained only a trace of it would be sufficient to cast grave doubts upon the normality of the element and lead one to ascribe an adventitious character to it. But in every case manganese has been abundant. The reactions for its identification are fortunately brilliant and decisive and at the same time indicate very well the relative amount of the element. The quantitative determinations show that the metal occurs in quite uniform amounts in the various specimens examined.

To summarize briefly the results: Some twenty-four analyses were made quantitatively upon material from about Madison. Some of these analyses were made upon single specimens of *Anadonta* or *Unio*, more were made upon a sample taken from the dried and pulverized tissues from a large number of specimens secured at one time from a given locality. Many of these analyses therefore represent the average of fifty or a hundred individuals. The average of the twenty-four analyses shows 21.8 per cent. of ash in the tissues, 4.52 per cent. manganese present in the ash and 0.95 per cent. manganese in the tissues. Mussels from the Wisconsin River averaged about 14.5 per cent. ash, 2.4 per cent. manganese in the ash and 0.35 per cent. in the tissues. From the Temagami Reserve of Ontario mussels averaged 15.4 per cent. ash, 3.1 per cent. manganese in the ash and 0.45 per cent. in the tissues. Specimens obtained from a great number of localities in Michigan, Illinois, Wisconsin, Indiana and Iowa average about 17.0 per cent. ash, 3.4 per cent. manganese in the ash and 0.60 per cent. in the dry tissue. A number of normal, average sized specimens from Lake Mendota were dissected into their more prominent tissues or organs. Analyses of these fractions gave the following results:

[1] Bradley, *Jr. Biological Chem.*, III., 151, 1907.

Tissue.	Per Cent. Ash.	Per Cent. Mn in Ash.	Per Cent. Mn in Tissue.
Muscle	6.0	4.87	0.293
Stomach; fibrous part	14.5	5.73	0.831
Stomach; non-fibrous part	32.0	4.66	1.492
Nephridial organs	27.0	5.31	1.434
Gills	33.5	4.89	1.638
Mantle	48.0	5.12	2.457
Liver	39.0	5.85	2.107
Eggs	37.0	2.024	0.749

Perhaps the most interesting result of these analyses was the presence of the element in the eggs and embryos, showing clearly that manganese is not an adventitious element picked up by the adult and held in the tissues from inability to excrete it—as, for example, iron compounds may be in the mammalian spleen after certain diseases involving great destruction of red corpuscles.

Another interesting point brought out in the above table is the exceedingly high mineral content of such a tissue as the mantle. Its ash content of 48.0 per cent. puts it in a class with mammaliam osseous tissue, though unlike the latter the mantle is soft and pliable. This is a type of tissue resembling no vertebrate organ or tissue of which we are aware. It seems probable that its function as a gland secreting the shell must have some connection with the high mineral content.

Having established as we believe the normality of the element manganese in the tissues of the Unionidæ, the question as to its origin naturally presents itself. It is hardly to be supposed that an animal of so great complexity as a lamellibranch would actually concentrate the element from its highly dilute solutions in lakes and streams. Such concentration is usually performed by lower forms of life which are then obtained as food. In the case of the Unionidæ the food origin of manganese is much more clearly apparent than is the food origin of copper in many of the hæmocyanin-bearing animals. The waters in which the mussels are found have invariably contained the brown masses of the manganiferous crenothrix mixed with diatoms, and other plancton forms which very probably contain manganese also. And this brown slime seems to be the normal food of these mussels so far as our observations extend. In Ontario

there are many lakes set in clean rocky basins, and fed by streams which leave little or no manganese stain on the rocks, and which appear to be free from the masses of crenothrix. In such lakes we have been unable to find mussels. In other lakes in the same region where seepage through glacial drift was apparent, or where the tributary streams flowed through such drift, discoloration of the stones, evidence of the presence of crenothrix, and the presence of the mussels seemed always to go together. For example in the Temagami Reserve, Lake Temagami itself is characterized by its clear water, and its clean rock basin. In certain parts occur limited areas of drift—sand and gravel—which are of insignificant amount. But though the bottom afforded, where the lake washed such drift areas, looked promising no mussels were found and the sandy stretches were apparently free from crenothrix. To the north of Lake Temagami are several lakes which lie in basins of glacial drift. In Sucker Gut, for example, sand and gravel beaches are abundant, the tributary stream flows through many miles of drift and carries enough manganese and iron in solution to stain its stones and pebbles strongly brown and black. The sand itself is stained with iron, and the brown slimy masses of crenothrix are abundant. In this lake and its tributary stream we found enormous numbers of small mussels wedged in thickly between pebbles or projecting from the sandy bottom. The many obvious examples in this region of the simultaneous presence of manganese, crenothrix and mussels, or of the absence of all three is probably more than a mere coincidence. We believe that more careful examination would show that the mussels require such manganiferous food as crenothrix and that they cannot live in waters where such food does not thrive.

In growing the mussels in aquaria the specimens always carry enough of the manganiferous organisms clinging to them so that in a few days an abundant development of the bacteria results. In this way several hundred grams of the dry organisms have been obtained for analysis. Such specimens are mixtures of a great variety of organisms and thus show large differences in chemical content. The ash content of such plancton crops vary from 24 to 76 per cent. of the dry weight; the manganese

from 0.13 to 1.84 per cent. of the dry weight. It has thus been possible to obtain, through the agency of these organisms, several grams of manganese from running water which contained about 0.0000066 per cent. of that element. The concentrating efficiency of these lower forms is therefore of a high order.

In secreting the shell, the Unionidæ deposit salts of manganese as well as of calcium and magnesium. The nacer of the shells, carefully freed from contaminating material, always gives a strong reaction for manganese; its presence in the shell is as characteristic as its presence in any of the tissues. It was thought therefore that an examination of fossil shells of the Unionidæ would be of interest in determining whether the manganese is of comparatively recent occurrence in these animals or whether it is a metabolic characteristic of long standing. So far we have had the opportunity to examine but one well preserved fossil shell. This was a specimen obtained through the courtesy of Dr. George Wagner who published a description of it in *Nautilus*, Vol. 18. The nacer of this shell was perfectly preserved, retaining its luster, though friable and crumbling to a powder easily. The fossil nacer gave 0.085 per cent. of manganese, while fresh shells of the present period frequently contain as much as 0.148 per cent. Thus it can be definitely asserted that the Unionidæ in pre-Pliocene times were using the element manganese as we find them today. It seems probable that the marine ancestors of the Unionidæ were themselves manganiferous. The fact that at least one marine lamellibranch is known makes such an assumption the more plausible.

To determine whether other marine lamellibranchs utilize manganese in this same way, an examination of the common forms along the coast of southern Massachusetts was made at the Woods Hole laboratory. In several forms the elements could usually be detected as a trace, but in such cases no import can be attached to its presence except as indicating that there is some marine low form of life serving as food for lamellibranchs, which also carries manganese. In *Pecten* the manganese was variable, sometimes large in amount, at others very small. It was frequently found abundant in the stomach contents. In *Modiola modiolus* the element was present in every specimen

examined, and it seemed to be rather uniform in amount. It was present in every tissue, and in the nephridial organs it was really abundant. In most of the tissues it was not at all comparable to the amount present in the Unionidæ, approximately 0.1 per cent. or less of the dry material. It will be remembered that the nephridial organs of *Modiola modiolus* are usually pigmented a dark brown—in all of some fifty specimens examined by us this brown pigmentation was prominent. It is possible that this lamellibranch deposits manganese obtained with its food in the nephridia in an attempt to excrete the element; that it is in this case adventitious and analogous to deposits of iron-containing pigment in mammalian tissues as the result of pathological conditions. It is interesting to note however that this marine mussel which stands morphologically fairly close to the Unionidæ, should appear to utilize the element so characteristic of the latter family.

It is our belief that other lamellibranchs will be found which, like *Pinna squamosa*, the Unionidæ and perhaps *Modiola modiolus*, utilize the element manganese in their metabolic processes. Such a chemical relationship may be useful in suggesting the lines of the evolutionary process which has led to the development of the present forms. It is our expectation to continue this line of investigation as opportunity permits.

THE SPERMATOGENESIS OF EUCHROMA GIGANTEA.

M. LOUISE NICHOLS.

The largest of the buprestid beetles, *Euchroma gigantea*, is native to Central and South America and is commonly found sunning itself on the trunks of trees. In such situations the beetles are not difficult to capture, as their movements are rather sluggish until they become thoroughly alarmed. The specimens from which the present study was made were taken at Culebra, Panama, in the month of August, at which time some of the beetles were mating, the male apparently attracting the female by a clicking sound produced by the elytra.

Upon sectioning the testes, I was surprised to find a complete series of stages from the spermatogonia to the mature spermatozoa, the younger stages not being confined to the larvæ or pupæ, as is frequently the case in insects. The testes were fixed in Gilson's mercuro-acetic-nitric solution or in Fleming's strong solution and stained with iron-hæmatoxylin or with saffranin and malachite green.

In the development of the germ cells of insects, as is well known through the results of the researches of Montgomery, Wilson, Stevens and others, there are present chromosomes which have been called heterochromosomes or idiochromosomes. Wilson (1909) has shown for the Hemiptera that in certain forms the idiochromosomes are equally well developed in both sexes, in others the male possesses one well developed, the other reduced in size, while in still others one is entirely lacking in the male. Stevens (1906) found somewhat similar conditions in the Coleoptera. Thus, the Elateridæ and Lampyridæ possess only the odd chromosomes, while the families Chrysomelidæ, Coccinelidæ, Scarabidæ, Silphidæ and Buprestidæ show one of the idiochromosomes reduced in size. In Carabidæ some members have an unequal pair of heterochromosomes, others an odd chromosome. *Euchroma gigantea*, as a member of the family Buprestidæ, belongs in the second of these groups (Figs. 21–22).

Besides the idiochromosomes, Wilson discovered in the Hemiptera a pair of chromosomes equal in size but noticeably smaller than the others, which he designated as *m*-chromosomes. According to the researches of Stevens these are occasionally present in the Coleoptera, *i. e.*, in *Trirhabda virgata* and *T. canadense* and in an unidentified buprestid. They likewise are represented in *Euchroma* (Figs. 19, 23, 24). In addition there are, in the spermatocytes, eleven chromosomes of more nearly equal size, making the total reduced number thirteen.

In most forms heretofore studied, the idiochromosomes are evident not only at the time of mitosis but also in the resting stage and prophases, for while the other chromosomes become resolved into the nuclear network, the idiochromosomes remain compact. It is in the manner of formation of the chromosomes during the prophases of the first maturation division and in the fact that neither at that time nor in the previous stages are the idiochromosomes distinctly different in behavior from the other chromosomes that the chief interest of the spermatogenesis of this beetle lies.

The nuclear network of the last generation of spermatogonia is of delicate texture. Chromatin masses occur at intervals, at first few in number and without constancy of position or shape (Fig. 5). The masses gradually become more distinct and form elongated threads near the center of the nucleus (Figs. 6, 7). The network breaks away from the nuclear wall and the synapsis is inaugurated (Figs. 8, 9). During this time there is no evidence of the idiochromosomes being isolated from the synaptic threads or failing to take part in their formation, nor, in the resting spermatocyte, do the idiochromosomes differ from the others. Stevens (1906) has reported a somewhat similar condition in the beetle *Tenebrio molitor*.

The nuclear network of the resting spermatocyte is more clearly defined than that of the spermatogonia and bears chromatin masses distributed with a fair degree of regularity (Fig. 10). This condition, however, does not continue. Instead of the usual spireme formation, the chromatin granules commence to migrate towards a specialized area within the nucleus (Figs. 11–14). The final result of this process is the formation of a

dense mass of chromatin in one part of the nucleus, the remainder of the nucleus being occupied by a fine network. It sometimes happens that more than one of these areas of aggregation develops (Figs. 15–18).

The outlines of the separate chromosomes may be seen, although they lie very close together. The nuclear network next begins to show a parallel arrangement of threads preparatory to the formation of the spindle (Figs. 17–18).

As is the case with other members of this order, the small heterochromosome is separated from the larger by the first division (Fig. 21). In mitosis the *m*-chromosomes tend to divide somewhat later than the others (Figs. 23–25).

After the second division the spindle fibers persist in the cytoplasm. They gradually cease to run parallel to each other, become more or less interlaced, and finally are arranged in spiral form (Figs. 26–28). Later they are converted into the tail of the spermatozoön (Fig. 32).

The chromatin of the spermatid at first condenses in large measure at the side of the nucleus nearest the spindle fibers, but as the latter lose their regularity of arrangement, the chromatin is dispersed throughout the network (Figs. 26–27). It next breaks into small fragments which migrate to the center of the nucleus, one mass, however, (probably the heterochromosome), remaining distinct from the rest (Figs. 29–32). As the nucleus approaches maturity, it elongates and the chromatin becomes finely granular, although fragments in chain form are still distinguishable (Fig. 33).

Discussion of Results.

A number of workers (see Blackman, 1903) have described "chromatin nucleoli." They occur in a wide variety of plant and animal groups and usually during a period of growth. In oögenesis and spermatogenesis they may be present in the oö- and spermatogonia as well as in the later stages. Among the groups in which they have been discovered are echinoderms, molluscs, arthropods, amphibians and mammalians, also in protozoa and plants. It may prove instructive to compare some of the more striking of these cases with that of *Euchroma*.

Sometimes the karyosphere is formed immediately after synapsis. Illustrations of this are furnished by the oögenesis of the dragonfly, *Plathemis* (McGill, 1906), and the pollen development of *Sarracenia* (Nichols, 1908). In this plant the karyosome is formed from the synaptic threads through an absorption of the chromatin substance by a nucleolus (Plate III., Fig. 1). The achromatic substance (linin) remains as a dense mass after the chromatin has passed by drops into the closely contiguous nucleolus. There is, therefore, a rather conspicuous separation here of chromatin and linin.[1]

In *Plathemis* the synaptic threads gather closely around the nucleolus and form what Miss McGill calls a double nucleolus (oxyphil+basophil). There is plainly an interchange of material between the two parts of the nucleolus and, as the network again expands, masses of basophil substance are elaborated within the nucleolus and pass out on to the network.

In *Euchroma* the transfer of chromatic material to the karyosome is less direct, inasmuch as the synaptic threads are first extended into the network of the resting spermatocyte (Fig. 10; Plate III, Fig. 4, *a*). The latter possesses no large nucleolus such as is present in the plant nucleus. Nevertheless a center of activity arises, towards which the chromatin passes and simultaneously also the linin, at first in the form of streamers radiating from the karyosphere (Figs. 11–12). Later, as the chromosomes become distinct within the karyosphere, the linin is incorporated with them (Figs. 16–18).

Blackman (1903) interprets the karyosphere of the spermatocytic prophase of myriapods as a mass of fine, granular filaments closely gathered about the accessory chromosome. As the nucleus approaches mitosis, the threads emerge from the karosphere, shorten and thicken to become the mitotic chromosomes (Plate III, Fig. 3, *a* and *b*). There is here no separation of chromatic from achromatic substance, only a strong attraction for both to a definite region of the nucleus. As compared with *Euchroma* this tendency manifests itself much earlier, for the karyosphere has already begun to resolve itself into definite chromosomes in

[1]A closely similar behavior of chromatin has been described for *Spirogyra* by Berghs (1906).

Scolopendra at a period when in *Euchroma* it is condensing (Plate III., Figs. 3 and 4). In *Scolopendra* there is likewise a karyosphere present in the spermatogonia, whereas it is lacking in *Euchroma*.

A somewhat different condition is described by Eisen (1900) for *Batrachoseps*. A karyosphere (chromoplast) is present in this object. At first it lies free in a vacuole, but later appears to attract "leaders" which might be compared to the radiating rays of *Euchroma* (Figs. 11–12).

Through these leaders small particles of chromatin are projected into the karyosphere and again emerge from it. The convergence of the leaders towards the karyosphere apparently corresponds to a synapsis stage, and as they finally break apart each chromosomal thread receives a portion of the karyosphere which is gradually distributed through the length of the thread[1] (Plate III., Figs. 2, *b* and *c*).

The condition in echinoderm eggs treated with Mg salts (Wilson, 1901) and in normal mouse eggs (Sobotta, 1895) is in some degree similar to that of *Euchroma*, for although there is apparently no separation of linin and chromatin, the karyosphere breaks up directly into the mitotic chromosomes (Plate III., Figs. 5, *a* and *b*).

What bearing have these facts on the question of the individuality of the chromosomes? In the paper on *Sarracenia* I suggested that the phenomena there "might be explained on the assumption that the morphological basis of the chromosomes remains in the linin while that portion of their substance which causes them to color deeply is absorbed by the nucleolus. If a similar interpretation be applied to the case of *Batrachoseps*, it will be seen that the linin retains its individuality more clearly than the chromatin, which may be transferred to the karyosphere. In the myriapods there is apparently no separation of chromatin and linin, but a tight coil of threads, consisting of

[1]Janssens (1905)in his later work on *Batrachoseps*, remarks: "il nous semblait très probable que les chromoplastes resultaient de la soudure très intime des chromosomes aux telophases à la faveur de rapprochement." "Il semble qu'une substance intensement siderophile, une sorte de nucleine, soit venue empâter tout le pole de la figure centre à ce moment. Il se peut qu'il s'agisse là d'un exsudat des chromosomes eux-mêmes."

both, forms the karyosphere. Possibly this might be regarded as a continuation and exaggeration of the synaptic condition, and, if this were true, a series of consecutive stages might be conceived between the typical synapsis and post-synapsis of most animals and plants and the extreme conditions presented by *Sarracenia*.

In the echinoderms, in *Mus* and in *Euchroma* a similar tendency to condense reappears and in the germ cells later than synapsis, owing, no doubt, to a chemical condition of the nucleus varying from the usual type. Here there is in one case (*Euchroma*) a more rapid condensation of chromatin than of linin and a consequent partial separation, while in the other (*Mus*, echinoderms) no such separation is apparent, chromatin and linin condensing simultaneously (Plate III., Figs. 4 and 5).

There is, however, little reason to believe that the difference in the method of formation of the spermatocytic chromosomes of *Euchroma* and most others insects is fundamental. The gradual change in coloration as resting chromatin becomes active goes to show that there is a chemical change in progress from less acid to more acid condition, accompanied by a condensation of substance. According to the differing constitution of different nuclei, this chemical activity might be confined to one center or distributed through the nucleus around several centers. If the latter were the case, the network of resting spermatocytes would break at various points and, condensing, form the chromosomes, but if there were but one center, the condensation would occur within a more circumscribed area. In *Euchroma*, while the latter method is more common, it may happen that the centers of condensation are multiplied (Fig. 15).

An interesting question concerning the relation of the chemistry of the nucleus to the individuality of the chromosomes presents itself at this point. If it be true that the aggregation of chromatin is accompanied by a decomposition of nucleo-proteids and a reduction of chromatin to nucleic acid or a simple compound of that acid and also true that the chromatin may be separated from the linin and gathered into a karyosphere, may it not be possible that the linin network is not homogeneous as regards its chemical character, but that in different areas are

developed different proteid substances which, when combined with the nucleic acid of the karyosphere, become active and colorable by chromatin stains. It may be that usually in the development of the germ cells the nucleic acid becomes chemically dissociated, but not visibly separated from these proteid substances, and consequently no karyosphere is present, simply chromosomes consisting of a condensed linin framework surrounded by nucleic acid.

PHILADELPHIA NORMAL SCHOOL,
June 4, 1910.

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Explanation of Plate I.

The drawings were made with the camera lucida, Zeiss microscope, oc. 12, obj. oil immersion 1/12. They have been enlarged to twice the diameter and reduced one half.

Figs. 1–4. Spermatogonia. Prophases.

Figs. 5–7. Last generation of spermatogonia. Prophases.

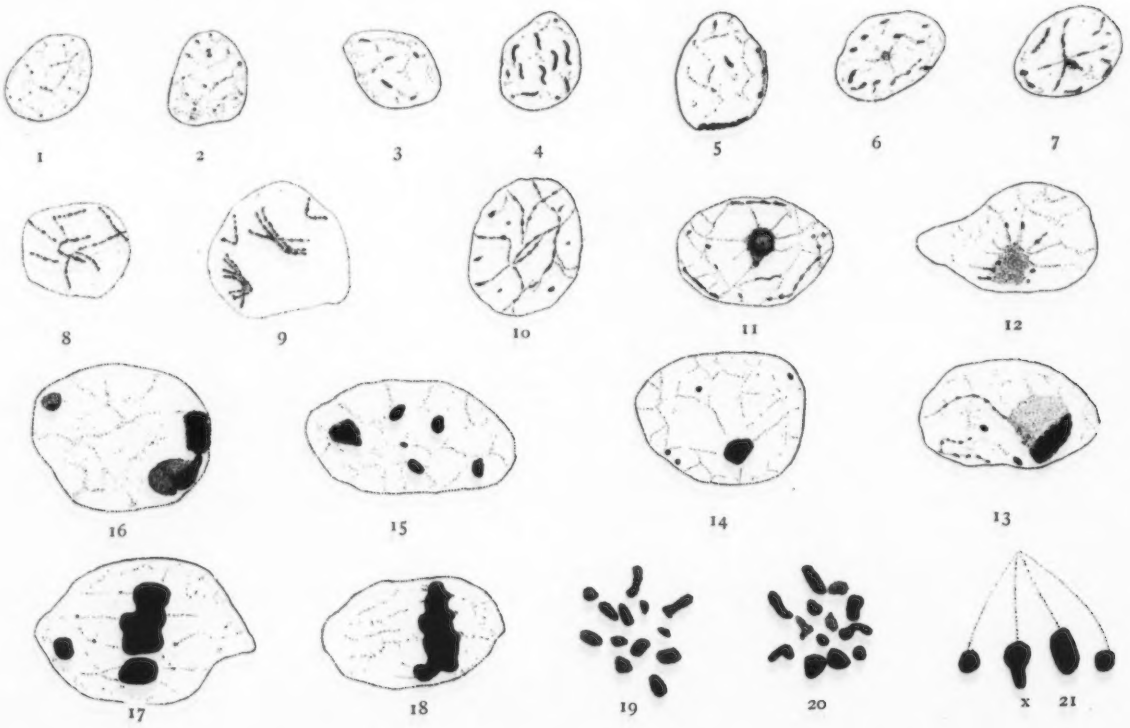
Figs. 8–9. Synapsis.

Fig. 10. Resting spermatocyte.

Figs. 11–18. Spermatocytes. Prophases of the first maturation division.

Figs. 19–20. Equatorial plates of the first maturation division. 13 chromosomes.

Figs. 21. The heterochromosome x.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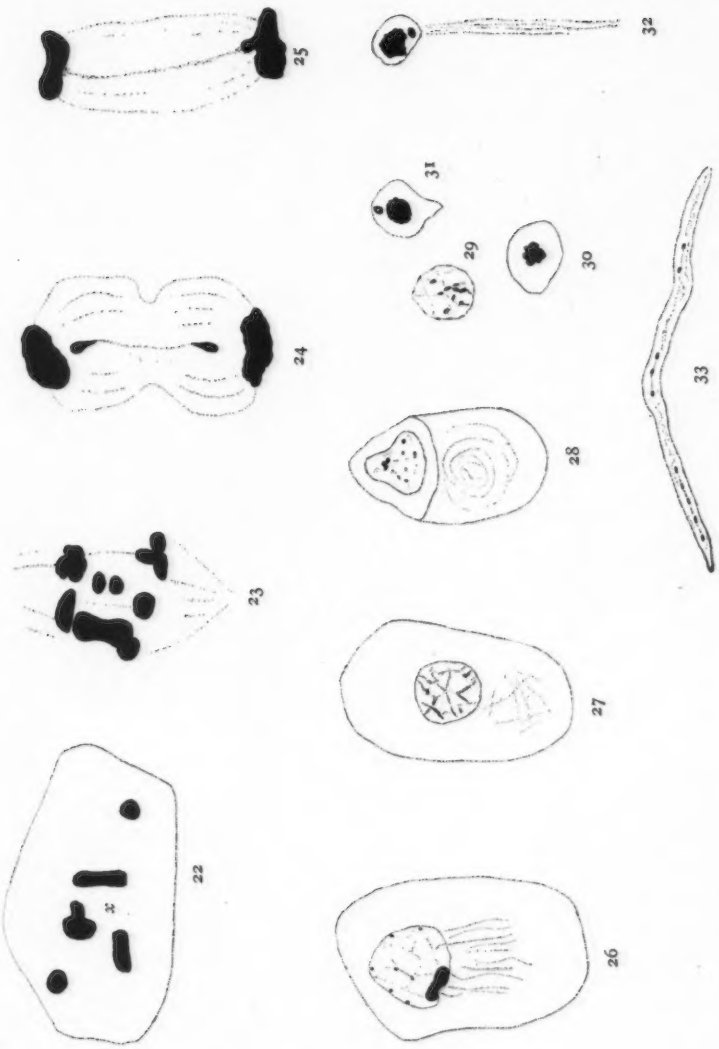
EXPLANATION OF PLATE II.

FIG. 22. The heterochromosome x.
FIG. 23. Side view of the first maturation division. Metaphase.
FIG. 24. Anaphase. Late division of the microchromosomes.
FIG. 25. Telophase. Traces of the late division of the microchromosomes.
FIGS. 26–33. Spermatids.

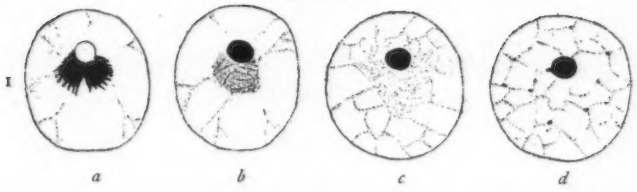


M. LOUISE NICHOLS.

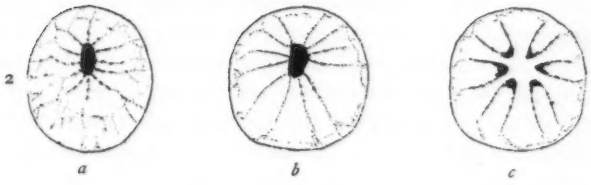
Explanation of Plate III.

Figs. 1–5. Diagrams showing the relation of the karyosphere to chromatin and linin.

Sarracenia.

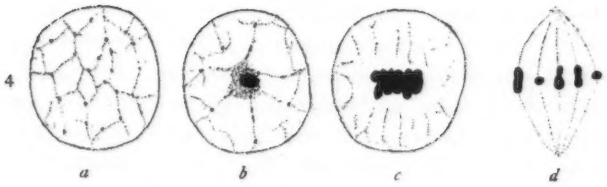


Batrachoseps.

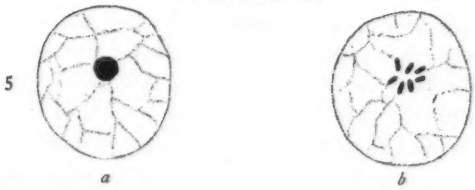


Scolopendra.

Euchroma.



Mus. Echinoderm.



M. LOUISE NICHOLS.

ON THE NATURE OF THE CANALICULAR APPARATUS OF ANIMAL CELLS.[1]

R. R. BENSLEY.

During the twelve years that have elapsed since the discovery by Golgi of the internal reticular apparatus of the nerve cell, much attention has been devoted by investigators to the consideration of similar reticular structures in cells. Our knowledge has progressed along several different lines, which have been determined more or less by the techniques employed by different investigators, and although all are not agreed as to the identity of the structures so revealed, their consideration together seems to be justified by the great similarity in configuration and location which these elements possess.

It is not necessary in this paper to review in extenso the literature on this topic, for that is well covered by the summary given by Holmgren ('02) in Merkel und Bonnet, Ergebnisse, etc., in which also the different theories of the nature of these structures are well brought out. The more recent contributions are considered in the article of von Bergen ('04) and that of Legendre ('08–'09). It will suffice here to summarize the progress that has been made along the different lines of investigation, and to consider the interpretations of these structures which have been advanced by different workers.

Golgi ('98) first described the internal reticular apparatus in the cells of Purkinje of the cerebellar cortex, where he demonstrated it by means of a modification of his well-known chrome silver impregnation method. He describes it as a closed net of fine fibers occupying the intermediate zone of the cell protoplasm and separated by a distinct interval from the nucleus on the one hand and from the surface of the cell on the other, that is to say, there was a zone of protoplasm on the periphery of the cell which was wholly free from the fibers constituting the network. Toward the nucleus the net sent out fine fibers so that the perinuclear space was not wholly devoid of fibers.

[1]From the Hull Laboratory of Anatomy, University of Chicago.

In his later studies Golgi confirmed this result for other types of nerve cells including spinal ganglion cells, spinal cord cells and cells of the cerebral cortex. In some of these cases he found the network provided with freely ending branches which terminated in a small swelling. In some cases, too, the fibers of which the network was composed had varicose swellings on them, and nodal enlargements, and, in some cells, he even found two concentric nets which differed inter se by the amplitude of the meshes and the size of the fibers. Golgi in all of his papers expressed himself with great reserve as to the nature of the network, but was confident that they had nothing to do with the neurofibrils and that they were not canals which had been filled with the silver precipitate. He was moreover certain that they were entirely intracellular and that they had no communication with extracellular structures.

In the meantime Golgi himself and his students had been extending the field of investigation to other than nervous tissues, and it had developed from these investigations that the reticular apparatus was not confined by any means to nerve cells but was present in a large variety of cells from different sources. For example, Negri ('00) demonstrated an apparatus of this sort in the cells of the pancreas and of the parotid gland of the cat, and in the thyroid epithelium of the dog. In these cells the situation of the network was quite characteristic and recalled the observations of Golgi that in young nerve cells with excentric nucleus the reticular apparatus was also excentric and located for the most part at one pole of the nucleus. In the epithelial cells, namely, it was found that the reticular apparatus was located near the nucleus, but between the latter and the free border on the lumen or surface, that is, it was distal to the blood vessels. Later, similar nets were found in the cells of the epididymus by Negri, in cartilage cells by Pensa ('01), and in striated muscle fibers by Veratti ('02).

The observations of Golgi were confirmed by a number of observers, using this method or one of the silver reduction methods of Cajal. Retzius, for example, obtained good impregnations of the apparatus in the nerve cells of the cat and rabbit, which corresponded in their salient characters with those of Golgi but

were not nearly so complete judging by the figures published. In view of this it is surprising to note that Retzius ('00) found, in some of the cells, the fibers of the network communicating by a branch with the surface of the cell, which Golgi had never observed in his more perfect preparations.

In a recent publication Golgi has adopted a new method for the demonstration of the reticulum which is based on the silver reduction method of Cajal. In view of this fact it is proper to mention here that Cajal ('07) has also studied the reticular apparatus in the nerve cell, which he accepts, contrary to Golgi, as a tubular apparatus to which he applies the name "Conduits de Golgi-Holmgren," thus accepting the interpretation of Holmgren that they are the same as the so-called juice canals described by him. He regards the appearances seen in his preparations as due to the presence of canals filled with a coagulable substance which has an affinity for colloidal silver. He also notes differences in the behavior of the apparatus in different animals, from which he concludes that the contents of the canals in different cells may have different chemical properties.

By the same methods as those employed by Cajal, Sanchez demonstrated an exceedingly interesting system in the striated muscle fibers of mammals and insects. In the former this system did not communicate with structures outside of the cell but sent free ending branches which terminated just under the sarcolemma. In the insects, however, he made the surprising observation that the intercellular network was in continuity with the tracheal system.

The second line of progress in the study of the reticular apparatus began with the discovery by Kopsch that it could be stained by prolonged immersion of the tissues in a two per cent. solution of osmic acid. In a short paper (Kopsch, '02) he described his method and contributed the results of his application of it. The results obtained corresponded very closely to those of Golgi but were obtained with greater certainty. Like Golgi he was unable to find any communications between the apparatus and the surface of the cell, although in addition to the osmic acid method he employed the resorcin-fuchsin method of Holmgren, to which reference will be made later.

The method of Kopsch has been exploited in particular by Misch ('03), and von Bergen ('04). The former found that the apparatus was not present in all cells and that in some cells it presented itself in the form of fragments, or of rows of granules. He found, moreover, in agreement with Golgi and Kopsch that the network never communicated with the surface of the cell, nor did it penetrate the nucleus. Von Bergen's studies extended to a very large category of cells ranging from wander cells to nerve cells. To show how general these structures are in animal cells a list of the elements in which von Bergen obtained positive results would have considerable interest. In addition to nerve cells he found a reticular apparatus in the following elements: prostate epithelium, pancreas cells, demilunes and mucous cells of the submaxillary gland of the cat, glandular epithelium from the trachea, chief cells of the fundus glands of the stomach, ciliated epithelium of the trachea, epithelium of the sweat glands, wander cells and many leucocytes, fixed connective tissue cells, cartilage cells, endothelium, smooth muscle, interstitial cells of the testis. The wide range of these observations taken in connection with the observations of Golgi and Cajal and their pupils indicate that the reticular apparatus is by no means a structure confined to a single cell category but is a cell organ of almost if not quite universal occurrence in the protoplasm of animal cells.

Before passing to a review of the investigations that have been made from the standpoint of the canalicular apparatus of Holmgren and others it may be of interest to refer briefly to the studies of Golgi on the development of the reticular apparatus. In the nerve cells of the fœtal calves of two or three months, he found the apparatus greatly reduced, often consisting of but a single fiber, with short branches running in various directions. In these cells the apparatus has a distinctly excentric position at one pole of the nucleus. In the new-born animal the net often extended around the nucleus, but left the perinuclear zone as well as the peripheral protoplasmic zone entirely free of such fibers. In old animals the apparatus was sometimes broken up into peculiar island-like fragments which however were connected with one another by single fibers. These observations suggest strongly that the apparatus constitutes a unit in its origin and developmental history.

The history of the intracellular canalicular apparatus, considered apart from the positive impregnations of Golgi and his followers, begins with the discovery by Holmgren ('99) of endocellular nets of juice-canals in nerve cells which he said was exhibited particularly well in preparations made from rabbit tissues. Almost at the same time Nelis ('99) described, in nerve cells fixed in sublimate or osmic acid and stained in iron-hæmatoxylin, etc., peculiar coil-like bands to which he gave the name "état spirémateux," the nature of which, however, remained to him fully obscure.

In a second publication (Holmgren, '99, 2) Holmgren described in greater detail the canalicular apparatus in the spinal ganglion cells of the rabbit, fixed in picric acid-sublimate and stained with toluidene blue and erythrosin. He found in these cells moderately fine canals of fairly uniform caliber which, anastomosing freely, formed a fairly dense network. The latter extended in general around the nucleus but often was found at one pole of the nucleus, more rarely at both poles. Here and there he found these canals communicating with pericellular canals, and at these points he was able to make out a distinct wall staining with erythrosin. He expressed the opinion that the canals were of lymphatic nature without however stating whether they were of extracellular or intracellular origin.

In 1899 Studnicka ('99) also described the canals in the protoplasm of the large ganglion cells of the trigeminus of *Petromyzon* and also in the spinal ganglion cells, in the nerve cells of the medulla oblongata and the cells of Reissner of the same animal. He explained the origin of the canals as due to the union of a row of vacuoles, and said that while many of the canals had smooth contours, yet in others might easily be seen the constituent vacuoles from which they had arisen. In a foot-note he remarked that he had not found in his objects the connection with extracellular structures described by Holmgren, although he admitted that the canals opened on the surface into the pericellular space.

In a series of papers dating from 1899 Holmgren has described the results of his investigations on this topic, covering a wide range of material including not only nerve cells, but cells from

various epithelia and from other sources. The existence of the canals has also been confirmed by a large number of observers including Kolster ('00), Fragnito ('00), Lugaro ('00), Donaggio (—), Pugnat ('01), Sjövall ('01), Smirnow ('01), von Bergen ('04) and others.

For comparison with the results of the Golgi and Kopsch techniques an enumeration of the different types of cells in which a canalicular apparatus has been found may be of interest. Holmgren demonstrated the canals in the following cells: gland cells of the pancreas and parotid, intestinal and gastric epithelium, epithelium of the epididymus, biliary duct epithelium, uterine epithelium, thyroid epithelium, liver cells, epithelium of the suprarenal gland. Retzius ('01) described similar canals in the giant cells of the bone-marrow, which, like Holmgren, he considered to be in direct connection with pericellular spaces.

It is to be noted that many of the objects studied by Holmgren coincide with those studied by Golgi and his pupils, and with those investigated by von Bergen, and that where this is the case, the canalicular structures described by Holmgren correspond closely in their location and in their configuration with those demonstrated by the other methods. Whatever conclusion we may reach with regard to the relation between the canalicular apparatus of Holmgren and the reticular apparatus in nerve cells, few who have studied the actual preparations made according to these different techniques in respect to epithelial cells and cartilage cells will deny their substantial identity. It is true that there are differences in the appearances obtained, but, in the opinion of many, these are sufficiently accounted for by the differences in the thickness of the sections studied in the different methods, and so, in the completeness of the apparatus which is brought to expression in a single preparation.

In his later papers dealing with these structures Holmgren has abandoned his original opinion that the canals are lymphatic in nature and constructed an entirely new hypothesis as to their nature. This hypothesis is based on the confirmation by him of the interesting observations of Nansen ('86) and Rhode ('91, '93, '95), that the nerve cells of certain Crustacea (Nansen, '86), and those of certain Gastropoda and Hirudinea (Rhode, *loc.*

cit.) were penetrated by a network derived from surrounding capsular cells. Holmgren found the nerve cells of *Helix pomatia* particularly suitable for the demonstration of these intracellular nets of capsular origin. He found here that the nerve cells were provided with a richer or poorer network of juice-canals, which were formed in the interior of a network of processes derived from other cells. He even found nucleated strands within the bodies of the nerve cell. In later publications (Holmgren, '01, '02, etc.)[1] he has developed this hypothesis on the basis of results obtained by the employment of a new method. He fixed his material in trichloracetic acid, or trichlorlactic acid, and stained it with a freshly prepared solution of Weigert's resorcin-fuchsin. By this method the protoplasm of the nerve cells stained faintly but that of the intracapsular cells stained dark violet, as did also the processes of the latter. By this means he was able to see processes of the darkly stained intracapsular cells which penetrated the nerve cells, branched within them, and anastomosed with one another, in order to produce an intracellular network. He applied this observation also to the nerve cells of vertebrates, and came to the conclusion that the latter were penetrated by processes of other cells which branched and anastomosed freely, to form in the interior of the nerve cells a "spongioplasma" which, however, in no wise belonged to the nerve cell, but was of extraneous origin. In the interior of these nets juice canals could arise, which communicated directly with similar spaces in the interior of the matrix-cells of this net. To this net of extraneous origin Holmgren gave the name "trophospongium." He regarded therefore the trophospongia not as fixed structures, but as undergoing a constant change, which depended upon the physico-chemical processes in the cell, and thought that, while at one moment the network of cell processes might sacrifice itself by liquefaction to the needs of the nerve cell, it might later be regenerated, by new growth of the process from without. He thus abandoned completely his former view that the canals represented circulatory or lymphatic structures, or a drainage system, in favor

[1]For complete references see Holmgren, E., "Neue Beiträge zur Morphologie der Zelle," in "Merkel-Bonnet Ergebnisse," Vol. 11, p. 275.

of the view that they represented the transitory phases of the reciprocal nutritive inter-relations of the capsular and nerve cells.

The object of the foregoing brief and incomplete résumé of the literature, has been to show that from three different lines of investigation we have evidence of the wide occurrence in animal cells, ranging in diversity from leucocytes to nerve cells and muscle cells, of a reticular apparatus, which exhibits itself in the form of a network of canals with colorless contents, or of a stained network according to the technique employed. The uniformity with which this apparatus has been discovered in those types of cells in which it has been sought justifies the expectation that similar methods will reveal similar structures in cells which have thus far not been investigated with this point in view. We are thus dealing with a cell organ of almost if not quite universal distribution in animal cells.

The question now arises—What is the significance of this structure?

The trophospongium theory of Holmgren, as far as I am aware, has found no support. Even if it were admitted for the nerve cells there are many categories of cells in which a reticular apparatus, or a canalicular apparatus is to be found, to which the theory is wholly inapplicable. For example, it is difficult to conceive how the reticular canalicular apparatus of the cartilage cells and of leucocytes, could be derived from the liquefaction of penetrating processes of other cells. Holmgren, it is true, has made an attempt to adapt his hypothesis to the canalicular apparatus of epithelial cells, and has described in the pancreas of the salamander the continuity of the intracellular network with intracellular strands which go to the periglandular connective tissue cells or to the centro-acinous cells. However, all of Holmgren's figures of preparations made by the trichloracetic acid, resorcin-fuchsin method are explainable on the basis of the canals having a precipitable content which when precipitated by the fixative, has an elective affinity for the dye. It is not by any means certain that the figures which Holmgren has given us of intracellular nets stained by fuchsin, in continuity with processes of capsular cells, do not really represent two different structures brought into apparent relation with one another by a common

affinity for the dye. It is certain, moreover, that the networks apparently composed of solid fibers demonstrated in the pancreas epithelium by the resorcin-fuchsin method, are not solid, for, in preparations made by this method, every cell in the section will show such a deeply stained network while sections of the same pancreas fixed in Kopsch's formol-bichromate solution and stained with iron-hæmatoxylin, will show in every cell a system of canals with unstained contents.

Accordingly, we must either reject Holmgren's hypothesis or assume that there are two sorts of these nets, those of cartilage cells and epithelial cells and leucocytes being different from those of nerve cells.

The statement of Legendre ('08–'09) that these structures are either wholly absent or are the result of pathological changes is not to be seriously considered, in view of the fact that this author in attempting to explain the positive observations in this regard of so many experienced investigators, is compelled to resort to the wholly unwarranted assumption that their results have been due to the selection of unhealthy animals, or to the fixation of tissues after several days of inanition, or several hours after death.

Many observers, including Retzius ('00), are inclined to believe that they represent an intracellular system of nutritive or drainage canals having direct relations with the lymphatic system. The extracellular communications are, however, denied by Golgi and his pupils, who have never seen them in their preparations made by the chrome-silver impregnation methods, nor by the silver reduction method. They are equally denied by von Bergen, who, however, admits the existence of canals in no wise connected with these, which he regards as artefacts, which do open on the surface of the cell.

Von Bergen ('04) who studied these structures by all three methods but, in particular, by the osmic acid method of Kopsch, agrees with Golgi that the structures are for the most part networks of fibers composed of a substance which reduces osmic acid, but explains the discontinuous elements found by him in many cells studied, by the assumption that they represent different stages in the formation or destruction of the apparatus

which thus would have a variable structure from moment to moment in the cell. He claims that the reticular apparatus arises by the appearance in the cell protoplasm of granules or droplets which arrange themselves in net-like or tortuous rows which fuse to form more continuous fibers, and further, that the network so formed can undergo vital changes, by virtue of which it loses its stainability and becomes dissolved, the canals so formed finally disappearing by absorption of their contents.

In view of the almost universal occurrence of these structures in all of the tissue cells of mammals, and in many of those of lower vertebrates and invertebrates, it seemed probable that they would not be wholly absent from the cells of the other great division of living organisms, namely from the cells of plants. Accordingly, I have studied with this end in view the structure of certain plant cells, using for this purpose in addition to the conventional methods of plant histology, those methods which in my experience were best for demonstration of the canalicular system in animal cells. It seemed probable, in view of the conditions found in the animal cell, that, if a homologue of the canal network of the animal cell were to be found in plant cells, it would be studied with greatest ease in those plant cells in which the vacuolar system had not yet reached its full development, namely in meristem tissues, sporogenous tissues and their products, cambium and embryonic tissues. The three last, however, did not lend themselves readily to this investigation because of the difficulty introduced by the slow penetration of the fixing agents, so that I have been obliged for the present to content myself with the results obtained in the root-tips of *Allium*, *Lilium* and *Iris*, and in the tapetum of the lily. Whether the consistent results obtained from the study of these cells are generally applicable or not to plant cells, future investigation will show. In the meantime, because of the fact that the results are at variance with the accepted views of the structure of the cells in question, because they furnish a new interpretation of the history of the vacuole of these cells and in particular because they seem to throw an interesting light on the question of the nature of the canalicular apparatus of animal cells, it seems wise to put these preliminary observations on record.

The descriptions which follow have been drawn largely from the study of preparations of the root-tip of the onion, but the observations made on the roots of the other genera mentioned are in full accord with them.

In his study of the vacuolar system of the cells of plants, Went ('88) describes the young cells of the onion root-tip as follows: "In the youngest cells of roots two or three millimeters in diameter I saw a great number of very small vacuoles; the largest had a diameter of four mikra, the smallest of one mikron." For these small vacuoles he claims reproduction by division, in the sense of the tonoplast theory of DeVries ('85). He also derives the vacuole of the older cell from these multiple vacuoles, by a process of coalescence.

In preparations made after fixation in Flemming's strong fluid, Hermann's fluid, Zenker's fluid, Carnoy's fluid, etc., and stained in iron hæmatoxylin, or in the three-color process of Flemming, results exactly corresponding to these were obtained, that is to say, the young cells contained a multitude of small vacuoles which by their coalescence seemed to form the large central vacuole of the older cell.

On the other hand, preparations made by methods which I had found to be most effective for the demonstration of the canalicular apparatus in animal cells, gave results which were wholly different. In these there was no trace in the youngest cells of the root tip of the multiple vacuoles described by Went and others, but instead, each cell possessed an intricate network of canals the component elements of which in the youngest cells were often of extreme fineness. These canals were best seen in the dermatogen cells on the surface of the root but were recognizable as such, though less well preserved, in the cells of the plerome. Fig. 1 shows a cell in which this system is composed of extremely fine canals. In this figure it will be seen that the canalicular system tends in these cells as in the animal cells to

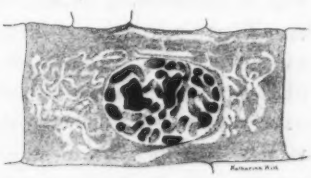


FIG. 1. Cell of outer layer of the root tip of onion, showing fine canals in the cytoplasm. × 800.

leave a peripheral zone of cytoplasm wholly free from the canals which constitute it. In Fig. 2 are shown four of the large wedge-shaped cells from the region of most active division of the dermatogen, in which the type of the canalicular system is well brought out. Here it will be seen that in these plant cells, as in the animal cells, the network tends to be concentrated on one side of the nucleus, and, as in the epithelial cells, this point of concentration is not one of the division poles of the nucleus but

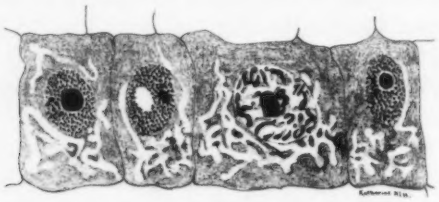


FIG. 2. Four cells of outer layer of root tip of onion, showing more advanced condition of the cytoplasmic canals. × 800.

corresponds to one side of the equator of the future spindle. The system constitutes a closed system of canals, lying in very close relation to the nucleus, never, however, invading, in dividing cells, the spindle territory. From this network run out branches which end freely often near the cell wall in a small expansion. Many of the canals in some preparations, and this is particularly true of the smallest canals, such as those shown in Fig. 1, show moniliform enlargements, as if they were on the point of breaking up into a row of vacuoles, or possibly, as if they had just been formed by the coalescence of a row of vacuoles. Again frequently, the canals show a spiral or tortuous course, as if they were fixed while in a condition of internal tension, which resembles very closely the spiral or tortuous condition found in many nerve cells (état spirémateux of Nelis).

Tracing this system in the older and older cells of the root tip it is found that as the cell retreats from the growing point, the canals become progressively larger and larger. In the intermediate stages of this process the condition depicted in Fig. 3 is obtained. Here there is still a continuous system of canals but they are fewer in number and broader than in the younger

cells. Ultimately by a continuation of this process we have the familiar picture of the plant cell with a large central vacuole across which run strands of protoplasm which are the last attenuated remains of the protoplasmic partitions between the canals.

A similar mechanism is revealed by the same technique in the tapetal cells of *Lilium candidum*. In preparations made after fixation in Flemming's fluid, the protoplasmic tip of the cell presents a foam-structure owing to the presence in it of a

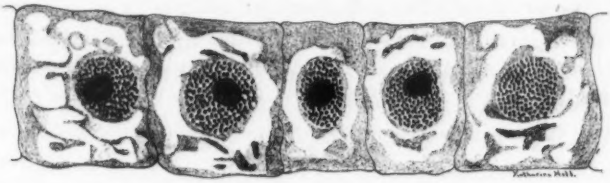


FIG. 3. Five cells of second row of root tip of onion, showing the expanded canals, beginning to form the large central vacuole.

large number of fine vacuoles. In preparations, however, which have been made by the technique referred to above, it is seen that instead of a multitude of minute vacuoles or alveolæ, there is a system of exceedingly fine canals forming a network which opens at intervals into the large vacuole which occupies the base of the cell.

Apart from the elements which constitute the tubes or vacuoles according to the method of preparation, the cytoplasm of these cells shows no indication whatever of an alveolar structure under the microscope. It is composed of an optically homogeneous ground substance in which are imbedded the mitochondria and other granular elements, for example plastids, which may be present.

The two different techniques, therefore, give us two entirely different conceptions of the history of the vacuole. According to the first the vacuole arises from the coalescence of preëxisting innumerable small vacuoles. According to the new methods, the vacuole in these cells constitutes a unit element from the very beginning, being represented in the younger cells by a single system of anastomosing canals.

The decision as to which of these views expresses the condition in the living cell must of necessity rest on the examination of living cells. Before taking up this question, however, we may discuss the significance of these observations in the interpretation of the canalicular apparatus of the animal cell for this interpretation does not of necessity imply the assumption that the canalicular structure so demonstrated in the plant cell has a real preëxistence in that form in the living cell. We may, on the contrary, treat the technique as an experimental method and discuss the results comparatively on this basis.

For the demonstration of the vacuolar system of plant cells as a network of canals I have found the following fixing fluids best adapted:

1. FORMALINE, BICHROMATE, SUBLIMATE.

Neutral formaline (freshly distilled)	10	c.c.
Water	90	c.c.
Potassium bichromate	2.5	gr.
Mercuric chloride	5	gr.

2. KOPSCH'S FLUID.

Potassium bichromate 2.5 per cent. in water	75	c.c.
Neutral formaline	25	c.c.

With these fluids, as indicated above, the cells of the root tip show a network of canals, whereas the same tissues fixed in Flemming's solution show, instead of canals, multiple small vacuoles. The same statement holds good for animal cells similarly treated. For example, the epithelial cells of the intestinal glands fixed in the formaline-bichromate-sublimate mixture, or in Kopsch's fluid, show a beautiful canalicular system, while the same cells fixed in Flemming's fluid show at the site of the canals merely a large number of exceedingly fine vacuoles. Thus whether we accept the multiply vacuolated condition, or the canalicular condition, as the preëxisting one in the living cell, the analogy between these structures in the animal and vegetable cell holds.

On the basis of the similarity in constitution of the canalicular apparatus of the plant cell to that of the animal cell, and of the similarity in behavior of this system when treated by the same methods and an account of the part these canals in the plant cell

take in the history of the vacuole of the latter, I think we are justified in stating for the present, to be sure, only as a working hypothesis, that the network of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system of the plant cell.

We may now return to the consideration of the question whether the canalicular system represents the true structure of the vacuolar mechanism of young plant cells or not. This question can, of course, be answered only by observations of the living cells themselves, and the investigation of these is beset by extraordinary difficulties in the case of the plant cell due in particular to the impossibility of finding a solution in which to examine the cells, which is not itself injurious. In the effort to find a suitable fluid for this purpose I tried solutions of potassium nitrate, of sodium chloride, and of cane sugar of different concentrations, but found in all that the surface layers of cells showed rapid changes of the structure of the protoplasm which made it difficult to study the presumably uninjured deeper layers of the sections. I was finally obliged to resort to the expedient of using as a mounting medium the freshly expressed juice of similar tissues, although even in this the cells of the surface layers of free-hand sections underwent more slowly the same change, In these surface layers so mounted the cells showed the multiple vacuolar condition described by Went. In the deeper layers, on the contrary, in sections of the onion root tip, one could see, with difficulty to be sure, but still unmistakably the canal system represented in Figs. 1, 2 and 3. As these cells are watched, however, the canals are seen to break up slowly into rounded vacuoles thus bringing about the condition generally recognized in these cells. I regard, therefore, the canalicular system as the true condition intra vitam of the vacuolar apparatus in these cells of the root tip, and believe that the multiply vacuolated condition is of secondary origin due in most cases to injury of the cell.

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A STUDY OF CHROMOSOMES OF TOXOPNEUSTES VARIEGATUS WHICH SHOW INDIVIDUAL PECULIARITIES OF FORM.

BARBARA HEFFNER.

INTRODUCTION.

The observations described in this paper were made during the winter and spring, 1909–10, in the Biological Laboratory of Bryn Mawr College.[1] Last November after my arrival in Bryn Mawr Professor Tennent suggested to me a study of the chromosomes in eggs of certain echinoderms with special reference to characteristic peculiarities of form, a question especially significant since the appearance of Baltzer's ('09*a*) paper on the chromosomes of *Strongylocentrotus lividus* and *Echinus microtuberculatus* which has thrown new light on the individuality of chromosomes in echinoids. Previous to this author's first publication on the subject ('08) there existed only a suggestion by Boveri ('01 and '07) that in some echinoids there occur chromosomes of a characteristic shape. Baltzer pointed out that in *Echinus microtuberculatus* there are two very long rod-shaped chromosomes, two long hook-shaped ones, and two or three horseshoe-shaped chromosomes, while in *Strongylocentrotus lividus* there are two long rod-shaped chromosomes, two long hook-shaped ones and in a part of the eggs one smaller hook-shaped chromosome. As this latter one occurs only in a part of the eggs—in about one half of them—Baltzer suggests ('09*a*, p. 596) that it is probably an idiochromosome whose smaller mate is one of the shorter rod-shaped chromosomes. The same suggestion is made in regard to one of the horseshoe-shaped chromosomes in *Echinus*, in cases where there are three of that type. While idiochromosomes and other heterochromosomes have for some time been known in insects, arachnids and myriapods,

[1]I take advantage of this opportunity to express my sincere thanks for the generous scholarship awarded to me by Bryn Mawr College and for encouraging suggestions from Professor Tennent and Dr. Stevens during the course of my work in their laboratory.

they have only quite recently been discovered by Gulick in the nematode *Heterakis* (Boveri, '10), by Baltzer in the echinoids, and by Guyer ('09*a* and *b*) in vertebrates.

Observations on metakinesis stages (Baltzer '09*a*, Plate XXXVII., Fig. 9) have shown that the hook-shape of certain chromosomes in *Strongylocentrotus* is due to the fact that the spindle fiber from each pole is attached at a point about one third of the length of the chromosome from one end, so that a shorter and a longer arm are formed. In the horseshoe-shaped chromosomes in *Echinus* the fiber is attached about half way between the two ends, so that the two arms are nearly of the same length (*ibid.*, Fig. 10). As for the origin of the hook-shaped and horseshoe-shaped heterochromosomes, observations on cross-fertilized eggs, $\frac{\textit{Strongylocentrotus}\ \male}{\textit{Echinus}\ \female}$, and on multipolar mitoses of *Strongylocentrotus* have shown that they come from the female pronucleus, the corresponding pair in the male being rod-shaped. Apparently the female has an unequal pair of heterochromosomes, one hook- or horseshoe-shaped, the other rod-shaped; while the male has a corresponding equal pair of rod-shaped chromosomes. It may be mentioned that this is the reverse of what is found in insects, but as in most insects the female nucleus must obtain more chromatin than the male nucleus.

Material and Methods.

My observations were made upon eggs of *Toxopneustes* and *Arbacia*, collected and preserved by Professor Tennent. As preserving fluid either picro-acetic or sublimate-acetic was used. Sections of 5 μ thickness were stained with Heidenhain's iron hæmatoxylin, except in a few cases mentioned later.

The figures are all drawn with Abbe's drawing camera, Zeiss oil immersion 2 mm. apochr. objective, oc. 12, enlarged to twice or four times the original diameter and reduced one half.

Arbacia punctulata.

The eggs of this species are quite unfavorable for detailed cytological studies. Not only are the chromosomes very small but the cytoplasm of the egg is filled with pigment granules so

that a sharp differentiation of plasma and chromosomes is impossible. Following Dr. Stevens's suggestion, I tried to bleach the section with H_2O_2, a method successfully applied in some other cases, but entirely useless in *Arbacia*. I therefore gave up further study of the chromosomes of this species.

TOXOPNEUSTES VARIEGATUS.

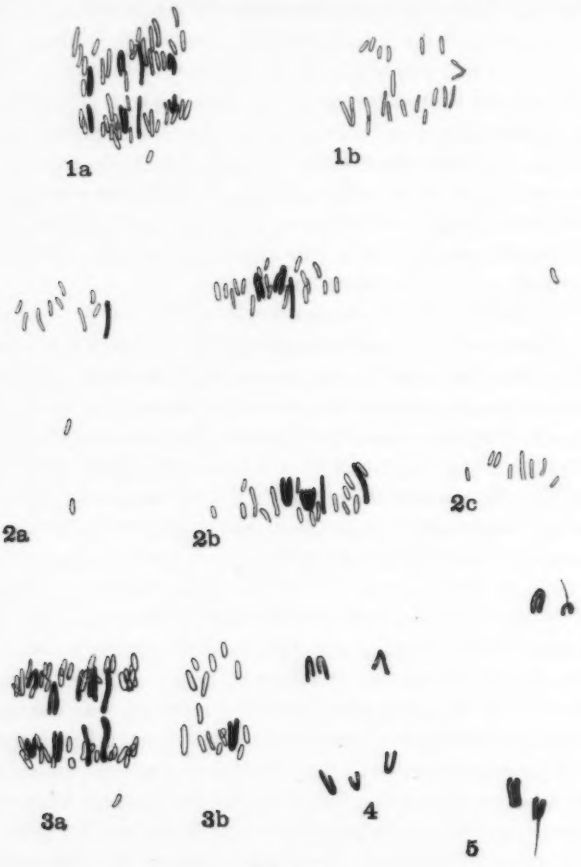
The observations were made on two series of eggs from two different localities, one from Beaufort, N. C., the other from the Tortugas. The results obtained are the same for both. I began with the study of the chromosomes in the first segmentation spindle and found in *Toxopneustes* as Baltzer ('09*a*) had done in *Strongylocentrotus* and *Echinus* a considerable variation in the length and form of the chromosomes.

As in *Echinus* and *Strongylocentrotus* there are two extremely long chromosomes in each daughter plate. Their behavior resembles that described by Baltzer in that these long rod-shaped chromosomes are often late in splitting and moving to the poles (compare Text-fig. 3, *a*, with Baltzer's '09*a*, Pl. XXXVII., Fig. 5, *a* and *b*). These chromosomes may also be seen in my Figs. 1, 2 and 3. Sometimes these long chromosomes are contracted more and then appear thicker and shorter (Fig. 1, *a*, the left pair of long rod-shaped chromosomes).

A type of chromosome of peculiar shape, found in all of the eggs, is one which is usually V-shaped, but sometimes more horseshoe-shaped (Figs. 4 and 5). Very frequently in late anaphases the two arms are parallel, or nearly so, and one may partly overlie the other, but there is hardly ever any doubt as to whether there is one chromosome with two arms present or two separate rod-shaped ones (Fig. 3, *a* and *b*). As may be observed from Fig. 4 the length of the two arms in these chromosomes may vary slightly when the arms are lying in one optical plane. I tried to determine whether this difference is confined only to certain chromosomes or to chromosomes in certain eggs, but no regularity seems to exist. The probable origin of the difference in length will be discussed later.

These V-shaped chromosomes occur in all fertilized *Toxopneustes* eggs and there are either two or three present. Among 34

one-cell stages examined with reference to this point, I found in 16 cases two, and in 18 three such chromosomes. Fig. 1, *a*, illustrates a case with two V-shaped chromosomes in each daughter plate. Their antagonistic position proves that they are division



FIGS. 1–5.

products of the same chromosome, their regular number, that they are not merely incidental features. The other chromosomes are very crowded, as most of them occur in one section. Their position has been slightly changed in cases where they were

over or under the V-shaped chromosomes, and this holds for all similar figures. Special care was of course taken to keep the position of the long rod-shaped and the V-shaped chromosomes in every case as accurately as possible.

Fig. 2, *b*, shows daughter plates of a late anaphase where there are three V-shaped chromosomes with more nearly parallel arms. Two of these chromosomes are very close together, one partly overlying another in each plate. Fig. 3 also shows in *a* and *b* three V-shaped chromosomes in early anaphase. One pair of these chromosomes appears smaller than the two others, but in examining other cases I found no regularity in the apparent size of the three pairs. Sometimes all three pairs vary a little, sometimes they are all of apparently the same size, sometimes only the members of one pair vary in apparent size. This difference may be due to difference in contraction or to an original difference in length of the chromosomes. As Baltzer's ('09*a*, p. 568) measurements of the hook-shaped chromosomes show, the length of chomosomes of a certain type is quite variable; it may vary from 9.75–14.0 mm. There is no resemblance however to the conditions in *Strongylocentrotus* in respect to the third pair of hook-shaped chromosomes which always is considerably smaller than the other two.

One of the three V-shaped chromosomes is probably a heterochromosome, as Baltzer assumes to account for the conditions found in *Strongylocentrotus* and *Echinus*. Studies in ovogenesis and spermatogenesis would be necessary to obtain evidence for or against the suggestion made.

As it is difficult to count the total number of chromosomes in a lateral view of the daughter plates I counted the number in polar views where fortunately the V-shaped chromosomes show very clearly.

Fig. 6, *a* and *b*, represents two succeeding sections through two anaphase daughter plates; the V-shaped chromosomes are the two double ones, finished in solid black. The number of chromosomes in each plate is 36. Fig. 7 shows a polar view with three V-shaped chromosomes; here also the number is 36.

I counted 14 anaphase plates from the pole, with a clear arrangement of chromosomes, and found the average number

36, always counting the two arms of the V-shaped chromosome as one.

Although I did not have very much material I was nevertheless able to trace the V-shaped chromosomes in the 2-, 4-, 8-, 12- and 16- to 32-cell stages.

FIG. 6.

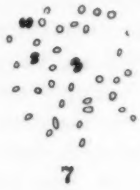
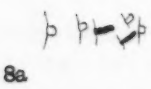


FIG. 7.

Fig. 8, *a* and *b*, shows a metaphase from a 2-cell stage, with the three V-shaped chromosomes distinguishable by their characteristic splitting figures, which are fully explained below.

Fig. 9, *A* and *B*, represents two adjoining cells of a 16- to 32-cell stage. In cell *A*, 9*b*, we see three V-shaped chromosomes



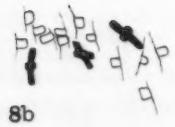


FIG. 8.

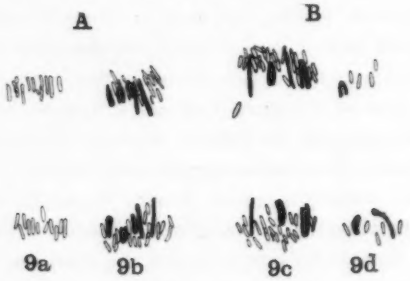
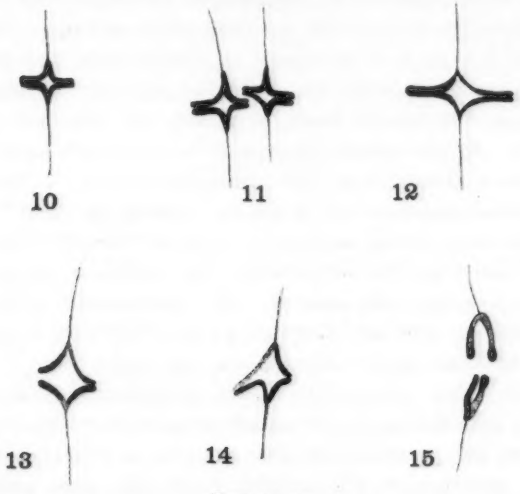


FIG. 9.

and the two long ones. In *B*, 9*c*, we see again two of the V-shaped chromosomes; and in 9*d* the third one. The two long ones are distributed between *c* and *d*. The chromosomes in this stage are so small and crowded together that an accurate count of their total number is impossible.

The figures 10–15, enlarged 4 diameters and in publication reduced one half, show splitting, or metakinesis, stages of the

V-shaped chromosomes. From these figures it is evident that the V-shaped chromosomes of the anaphase come from a long rod-shaped chromosome to which the spindle fibers are attached with more or less regularity in the middle of the chromosome (Fig. 12). If the separation of the daughter-chromosomes takes place earlier at one end than at the other (Fig. 13) or one arm of the chromosome is contracted more than the other, or, as Fig. 14 suggests, if some hindrance on one side prevents one



FIGS. 10–15.

end from moving toward the pole as rapidly as the other end, then the variations in the length of the arms mentioned above (Fig. 4) appear. In comparing these figures with Baltzer's '09*a*, Pl. XXXVII., Fig. 10, we cannot fail to find a close resemblance.

In Baltzer's paper '09 *a*, p. 607, we notice a suggestion that in the hook-shaped chromosomes there may be a union of two chromosomes, end to end at the point where spindle fibers are attached. The reasons given for this suggestion are the extraordinary length of the hook-shaped chromosomes, and the fact that all other chromosomes are attached to the spindle fibers by one end. This suggestion is a very natural one, for such apparently homogeneous but plurivalent chromosomes are

known in *Ascaris*, and compound chromosomes are found in the maturation mitoses of certain insects (McClung, '05, Payne, '09). In some of these latter cases (Payne, '09) the plurivalent chromosome has a spindle fiber attached to each unit in the early metaphase and in many cases two spindle fibers from each pole are attached to one of each of the four units of a tetrad in primary maturation mitoses (Stevens, '10).

Since the V-shaped chromosome of *Toxopneustes* seems to be exactly comparable to the hook-shaped chromosome of *Echinus* and *Strongylocentrotus*, the question arose whether, assuming that the V-shaped chromosomes of *Toxopneustes* may be bivalent, one might by careful observation be able to trace in the early metaphase two spindle fibers from each pole attached to each of them. As the spindle fibers were not especially clear in the preparation stained with iron-hæmatoxylin alone, a few slides were counter-stained with Rubin S. Among 42 cases I found only two where I was inclined to count two fibers; in all other cases I was certain that only one fiber from each pole was attached to each V-shaped chromosome. My observations have therefore failed to add any facts supporting Baltzer's suggestion, which, however, future investigation may verify.

Baltzer ('09*a*) traced the heterochromosome in *Strongylocentrotus* and *Echinus* to the female pronucleus. Unfortunately I was not able to obtain suitable material for this purpose, but further investigation will probably reveal the same conditions as in *Echinus* and *Strongylocentrotus*.

Discussion.

Comparing the chromosomes with peculiar shape in *Echinus* and *Strongylocentrotus* with those in *Toxopneustes* we find that the hook-shaped chromosomes in *Echinus* and *Strongylocentrotus* have no exact equivalent in *Toxopneustes*. The two extremely long rod-shaped ones are found in the three species. The V-shaped chromosomes in *Toxopneustes* are very similar to the horseshoe-shaped chromosomes of *Echinus* in respect to their formation and the equal length of their two arms. They differ from the *Echinus* chromosomes as already mentioned in their length and slenderness. As in *Echinus* we are not able to

distinguish a particular one of these three V-shaped chromosomes as a heterochromosome.

The discovery of individuality of form among the chromosomes in echinoids is a very valuable factor in support of Boveri's "Individualitätstheorie" of the chromosomes. One also welcomes every such means of distinguishing parental chromosomes in cross-fertilized eggs. *Toxopneustes* for instance has been used for cross-fertilization (Tennent, '07 and '10). These chromosomes which show marked individuality of form will be of special value in cases of cross-fertilized eggs, where, as shown by Herbst ('09) and Baltzer ('09*b*), the chromosomes of one parent are almost entirely eliminated during the first segmentation divisions.

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ON THE INHERITANCE OF COLOR IN THE AMERICAN HARNESS HORSE.

A. H. STURTEVANT, JR.

In a study of the English thoroughbred horse C. C. Hurst[1] has shown that chestnut is recessive to bay and brown. He supposes that the presence of black in the coat is the dominant character. Now black, gray and most roan horses also have black in their coats, but 95 per cent. of the English thoroughbreds are bay, brown or chestnut, so that Hurst was unable to verify his supposition. The American trotting and pacing horse, however, a close relative of the English thoroughbred, exhibits colors in proportions much more favorable for an investigation of this kind. These proportions are about as follows: bay, 53 per cent.; black, 13 per cent.; brown, 15 per cent.; chestnut, 14 per cent.; gray, 3 per cent.; roan, 2 per cent.; dun, .1 per cent.

Perhaps before going further it will be well to give a brief discussion of these colors. According to Miss F. M. Durham, as quoted by W. Bateson,[2] there are three pigments, yellow, black and chocolate, concerned in the color of horses, as in mice, rabbits and other animals. Chestnuts have the yellow pigment alone.[3] Bays have both yellow and black pigments, and browns are only very dark bays, shading into the self-colored blacks on the other extreme. Grays have black hairs mixed with white ones, usually in a dapple pattern. Roans are of at least three types. The most common are the bay, red or strawberry roans, which have yellow-black hairs intimately mixed with white ones. The black, blue or gray roans appear to differ from grays chiefly in that their black and white hairs are more intimately mixed. The chestnut roans have yellow and white hairs. As will appear later the fact that there is no black in this class introduces a possible source of error into my calculations. However, these chestnut

[1] *Proc. Royal Soc.*, Vol. 77, B., 1906, p. 388.

[2] "Mendel's Principles of Heredity," p. 125.

[3] According to Bateson some chestnuts are really chocolates, but these are like the yellows in having no black.

roans are rare, forming something less than 10 per cent of the total number of roans seen on the streets of New York, and nearly all of those seen are heavy draught horses, so that I feel sure they are very rare indeed among blooded trotters. The official records do not distinguish between these three types of roans, but in the journals it is not rare to see a horse described as belonging to one of the two commoner classes, though I have never yet seen in them a reference to a chestnut roan. There are several types of duns, but, as appears in the list above, all are rare. In a few families duns seem to be dominant to bay, brown and black, and one was connected with gray, but beyond this I have found nothing about the color. Lastly there are a very few spotted trotters, but these are all poorly bred ones, with short pedigrees, and I have done nothing with them.

I have tried to show here that Hurst's discovery of the dominance of bay and brown to chestnut holds good for the American harness horse, and that gray, black and roan, or all other colors containing black, are also dominant to chestnut. In order to avoid confusion I shall call this dominant factor for the presence of black Hurst's factor. Apparently all trotters have the factor for chestnut, which I shall represent by *C*. This factor is hypostatic to all the others here mentioned. The factor next highest in the scale is that for black, or Hurst's factor, *H*, its absence being *h*. Next higher is that for bay, *B*, its absence being *b*. At the top stand the gray and roan factors, *G* and *R*. Now most horses have neither of these last two, and are therefore *ggrr*. A chestnut will always be *Chh*, but may have any combination of the other factors and their absences, since they produce no visible effect in the absence of Hurst's factor. Self blacks are *CHHbb* or *CHhbb*, since bay is epistatic to self black. Bays have one or two *C*'*s*, *H*'*s* and *B*'*s*. Grays have *C*, *H* and *G*, and roans have *C*, *H* and *R*. Whether these last two must have *B* or not is not clear. I shall discuss the three epistatic colors more fully and give my theory as to brown when I have presented the evidence as to Hurst's factor.

The chief authorities for the statistics and color pedigrees given here have been Wallace's "Year Book of Trotting and Pacing" and Wallace's "American Trotting Register," both of which are official records.

It is a recognized fact among the breeders of harness horses that certain stallions never produce chestnut foals. In *Wallace's Monthly* for February, 1880, there is an article by "Truth," in which he says: "I have learned that neither of the brothers [Volunteer and Sentinel] have ever sired a chestnut colt." W. H. Marrett, in the September, 1890, issue of the same paper, tells us that the two bay sires Volunteer and Electioneer never had chestnut foals. Both were by a bay sire (Rysdyk's Hambletonian, which appears in the first table below), one being from a bay mare, the other from a brown. In a sale catalogue issued in 1903 C. W. Williams says of the brown stallion Belsire: "His get are . . . bays, browns and blacks." This horse is a son of the Electioneer mentioned above and of a black mare whose sire was a black and dam bay. He is a full brother to the bay Chimes which appears in the table below, and to Bow Bells, bay, and St. Bel, black, both also probably homozygous.

I have found a good many sires homozygous for Hurst's factor. The small number of gray and roan sires in the table below is to be explained by the small number of those colors existing. It will be noticed that two of the number have one chestnut foal each recorded. Director's was found in an advertisement in a horse journal—obviously a poor authority, as the pedigree might easily have been false. That by Jay Bird is Cardenas, trotting record 2:26¼, from a chestnut mare. He is recorded as a chestnut by the "Year Book." But the "Year Book" does sometimes make mistakes in the matter of color. Among others I could mention is the case of the bay stallion Charley Wilkhurst, recorded as a gray gelding.[1] In this connection it is worth noting that Hurst found about 1 per cent. of exceptions recorded in his investigation, but was able to explain most of them by showing them to be probably mistakes.

[1]See *The Horse Review* for December 12, 1905, p. 1424.

TABLE OF SIRES HOMOZYGOUS FOR HURST'S FACTOR.

Stallion.	Color.	Sire's Color.	Dam's Color.	Foals.						
				Bays.	Blacks.	Browns.	Chestnuts.	Grays.	Roans.	All.
Chimes[1]	bay	bay	black	15	1	4	0	0	0	20
Electioneer[2]	bay	bay	brown	39	2	9	0	2	0	52
Happy Medium	bay	bay	bay	59	0	4	0	4	2	69
Rysdyk's Hambletonian	bay	bay	brown	40	0	8	0	2	0	50
Director	black	brown	bay	15	35	19	1	2	2	74
Gambetta Wilkes[3]	black	brown	brown	12	12	6	0	0	0	30
Oro Wilkes	black	black	black	15	3	4	0	0	0	22
Sable Wilkes	black	bay	black	23	19	7	0	0	0	49
Dictator	brown	bay	black	12	7	5	0	0	0	24
George Wilkes	brown	bay	brown or roan	12	5	3	0	0	1	21
Prodigal	brown	bay	bay	94	0	19	0	3	0	116
Conductor	gray	bay	gray	3	1	5	0	15	0	24
Pilot Medium	gray	bay	gray	49	0	5	0	61	1	116
Eagle Bird	roan	roan	brown	15	1	4	0	0	8	28
Jay Bird	roan	brown	roan	54	0	15	1	3	63	136
Margrave	roan	brown	roan	28	0	5	0	2	30	65

[1]Half of these foals from mares by the chestnut Mambrino King.

[2]Twenty-nine of these chestnut mares.

[3]Eleven of these from chestnut mares.

I have found only 69 cases of chestnuts being mated together, but in all these the result was chestnut. They are from the following stallions:

Chestnut Sire.	Color		Number of Chestnut Foals from Chestnut Mares.
	Sire.	Dam.	
Nutwood	bay	gray	16
Robert McGregor	bay	bay	7
Attorney	bay	?	6
Daniel Lambert	bay	?	5
Elyria	chestnut	black	4
Hinder Wilkes	bay	chestnut	4
Mambrino King	black	?	4
Six different sires with two each			12
Eleven sires with one each			11
Total			69

The finding of foals from heterozygous sires and chestnut mares is a very slow and laborious task, but I have found some from the following stallions:

Stallion.	Color.	Foals from Chestnut Mares.	
		Chestnuts.	Total Found of all Colors.
Alcyone	bay	3	7
Axtell	bay	8	10
Boreal	bay	6	8
Guy Wilkes	bay	7	8
Norval	bay	1	6
Onward	bay	8	20
Red Wilkes	bay	8	14
Strathmore	bay	3	6
Total bay sires	bay	44	79
Grattan	black	1	4
Simmons	black	5	7
Total black sires	black	6	11
Alcantara	brown	2	8
Allerton	brown	8	17
Total brown sires	brown	10	25
Alcryon	gray	2	2
Re-election	gray	3	4
Total gray sires	gray	5	6
Grand Total		65	121
Expectation		60½	121

Here again I have been handicapped in working with gray and roan by the small number of sires of those colors, and also by the fact that most of the best known of those existing seem to be homozygous. However, I have found some sires of those colors which throw a fair percentage of chestnut foals, as shown in the following table, which shows all known foals. Two blacks are also included.

Stallion.	Color.	Chestnut Foals.	Foals of All Colors.
Bellini	black	3	17
Mambrino Patchen	black	3	16
Alcryon	gray	9	30
Pilot, Jr.	gray	4	20
Jay Hawker[1]	roan	1	20
Roan Wilkes	roan	3	10
Tom Hal, Jr.[2]	roan	1	15

[1] The one chestnut from Jay Hawker is scarcely to be doubted, as he is Country Jay 2:07¼, world's champion trotter under saddle, and one of the most prominent race horses of the season of 1909.

[2] This one chestnut also is not a doubtful one. His name is in fact Chestnut Hal.

It seems to me that we have here sufficient proof that Hurst's original supposition is right—that the dominant character is the black in the coat. However his idea that the black, if present, exists on the fetlocks, seems to me to be unjustified, as Joe Patchen, a heterozygous black, has white feet, though the white does not reach as far up as the fetlock on the left hind one. Grattan, another heterozygous black, also has three white fetlocks. It is not at all rare to see pictures of bays or browns with one foot or more white as far up as the fetlock or further. Here are some examples: bays—Capo, both hinds; Moko, right hind; Ario Leyburn, left hind; Allerworthy, both lefts; Hail Cloud, right front; browns—Redlac, both hinds; The Harvester, left hind; Searchlight, both hinds. At least one of these (Moko) is homozygous.

In the case of the next factor, bay, a complication arises in regard to brown. As explained before the presence of bay is dominant to its absence, and the color next below it in the scale is black. Brown is a color between these two, shading into both extremes. It seems that brown is usually a heterozygous color, represented by *CHBb*, but that bay also is quite often heterozygous, and that brown may occasionally be *either* of the homozygous types, *CHBB* or *CHbb*. This suggests the idea that the line between black and bay should be drawn somewhere near the black limit of brown. The obvious result of this complication is the creation of considerable confusion in the numerical proportions of the two colors. It is evident that, except for this complication and the appearance of some chestnuts, bay will act as though it were an ordinary dominant to black.

Below is a table showing twelve sires homozygous for the bay factor. Six of them are bays, and one is the only brown certainly known to be homozygous for this factor. Two are chestnuts, and therefore lack Hurst's factor. The other three are a gray and two roans, and it will be noted that all three of them appeared in the table of sires homozygous for Hurst's factor. They are not bays because they also bear other factors, as I shall explain later. Of the bays two are homozygous and four are heterozygous for Hurst's factor. The single black from Robert McGregor is Bobby Good, pacer, 2:11¼, out of a daughter of Ashland Wilkes.

Stallion.	Color.	Sire's Color.	Dam's Color.	Foals.						
				Bays.	Blacks.	Browns.	Chestnuts.	Grays.	Roans.	All.
Ashland Wilkes	bay	bay	?	90	0	0	19	5	0	114
Rysdyk's Hambletonian	bay	bay	brown	40	0	8	0	2	0	50
Happy Medium.	bay	bay	brown	59	0	4	0	4	2	69
Onward.....	bay	brown	bay	85	0	7	24	1	0	117
Red Wilkes.....	bay	brown	?	92	0	7	22	3	0	124
Sphinx.....	bay	bay	chestnut	30	0	5	9	4	0	48
Prodigal.....	brown	bay	bay	94	0	19	0	3	0	116
Axworthy.....	chestnut	bay	bay	33	0	2	32	1	4	72
Robert McGregor....	chestnut	bay	bay	42	1	1	30	2	1	77
Pilot Medium ..	gray	bay	gray	49	0	5	0	61	1	116
Jay Bird	roan	brown	roan	54	0	15	1	3	63	136
Margrave	roan	brown	roan	28	0	5	0	2	30	65

The mating together of blacks should produce only blacks and a few chestnuts, since chestnut is the only color hypostatic to black. However it does produce some browns and is recorded as producing occasional bays. The bays so far found are: Kipling 2:21¼, by Gambetta Wilkes ex Margaret W., and Gipsey Bel 2:30, by St. Bel ex Gipsey A. Now there is some reason to doubt the color of St. Bel. His dam was the black Beautiful Bells, but his sire was the bay Electioneer, and he is the only black among the eleven foals by Electioneer from black mares that I have found. Moreover, counting St. Bel, I have found only two black foals out of a total of 52 from Electioneer. Since Electioneer was homozygous for Hurst's factor this small proportion cannot be partly explained by supposing that more of the 52 would have been black had they had that factor. Neither is it possible to suppose that the small proportion is due to suppression by the gray or roan factor, since the 52 include only two grays and no roans. It looks very much as though Electioneer were homozygous for the bay factor. St. Bel died young, leaving few foals, so that I have been unable to get much data about his descendants. As to the other apparent exception, Kipling, I shall only call attention to the fact that neither he nor his dam are very well known. The following table shows foals from two black parents.

The case of foals from heterozygous sires and black mares is

Black Sire.	Foals from Black Mares.			
	Bays.	Blacks.	Browns.	Chestnuts.
Bellini	0	2	0	1
Gambetta Wilkes	1	3	0	0
Grattan	0	3	0	0
Patchen Wilkes	0	8	0	0
Sable Wilkes	0	7	1	0
Simmons	0	3	0	0
Nine others	1	8	3	0
Total	2	34	4	1

the one where the uncertainty about brown causes the most trouble. Since the presence of Hurst's factor is necessary before any of the three colors in question can appear I have left out the chestnut foals in the following table of foals from black mares.

Stallion.	Color.	Foals from Black Mares.		
		Bays.	Blacks.	Browns.
Axtell	bay	1	4	3
Guy Wilkes	bay	1	7	3
Alcantara	brown	1	3	0
Allerton	brown	0	3	3
Nutwood	chestnut	2	3	0
Total		5	20	9

The numbers in the above table are small, and, as in the similar case with Hurst's factor, I have supplemented it with one giving all known foals, chestnuts being again left out.

Stallion.	Color.	Foals.				
		Bays.	Blacks.	Browns.	Grays.	Roans.
Bow Bells	bay	8	2	0	0	0
Directum Kelly	bay	7	2	2	0	0
McKinney	bay	14	4	8	0	2
Baron Wilkes	brown	44	10	28	0	1
Brown Hal	brown	34	10	15	2	4
Dictator	brown	12	7	5	0	0
George Wilkes	brown	12	5	3	0	1
Highwood	brown	24	5	8	0	0
American Star	chestnut	6	2	1	1	0
Mambrino King	chestnut	4	3	1	0	0
The Earl	chestnut	2	1	1	0	0
Conductor	gray	3	1	3	8	0
Pilot, Jr.	gray	3	2	1	10	0
Eagle Bird	roan	15	1	4	0	8

Now to turn to the gray factor. In the first place, I make no claims that all gray is epistatic to the four usual colors. Perhaps

it will be best to take up the grays by families, and I will first treat of those in which it is epistatic, and then of the one in which it seems not to be.

Most of the high-bred grays of to-day go back to Pilot, Jr., through an unbroken line of grays. This horse was a gray, son of a black sire and of a mare of untraced breeding whose color I have been unable to find. The gray sires in the next table all get their gray from him. This table includes all known foals except chestnuts, these being omitted for the same reason as in the last case. Since gray is an unpopular color it is safe to say that nearly all these foals were from recessive (*gg*) mares. I have so far found only one case of grays being mated together, and, since the produce of that mating was never heard from after racing, I know of no horse homozygous for the gray factor, *G*.

Gray Stallion.	Sire's Color.	Dam's Color.	Foals not Gray or Chestnut.	Gray Foals.
Bayard	gray	?	4	5
Pilot, Jr.	black	?	5	10
Pilot Medium	bay	gray	55	61
Re-election	bay	gray	12	15
Total			76	91

The following sires are all sons of gray members of the Pilot, Jr., family. Several of them have gray foals in this table, but all of these are from gray mares.

Stallion.	Color.	Foals				
		Bays.	Blacks.	Browns.	Grays.	Roans.
Lord Russell	bay	16	0	0	1	0
Peter the Great	bay	24	1	2	2	0
Darknight	black	5	2	2	0	0
Electricity	brown	10	1	7	1	0
Expedition	brown	46	3	10	5	0
Highwood	brown	24	5	8	0	0
Mambrino Russell	chestnut	6	0	3	0	0
Nutwood	chestnut	70	9	2	3	0

There can be little doubt that in the Pilot, Jr., family gray is an ordinary dominant, and there are other families where it seems to be, though there is not as much evidence. One of these goes back to the mare Sontag Mohawk, and through her probably to imported Messenger, the foundation of the breed of American

harness horses. Another goes to the mare Bashaw Belle, daughter of Young Bashaw, gray. Of the three horses below, Conductor is a son of Sontag Mohawk, Manager is a grandson of Bashaw Belle, and Alcryon is out of Lady Blanche, daughter of Privateer, both grays.

Gray Stallion.	Sire's Color.	Dam's Color.	Foals not Gray or Chestnut.	Gray Foals.
Alcryon	bay	gray	14	7
Conductor	bay	gray	7	8
Manager	chestnut	gray	11	12
Total			32	27

Eros and Walnut Hall, a brother and son, respectively, of Conductor, and both browns, have no gray foals among the 35 I have found.

There is one family in which gray appears not to be epistatic. The first member of the family that I know of is General Wilkes, gray, son of George Wilkes (an ordinary brown which appears in two of the tables already given and has nine different sons in them) and of a gray mare. This stallion had some gray foals, of whose descendants I have found no record. But he had two sons not gray which have produced many gray foals. They are Dispute, black, and Bobby Burns, bay. I am not sure of the color of the dams of any of Dipute's gray foals, but in the case of Bobby Burns some of them are from bay mares. Dispute's maternal color pedigree I do not know, but Bobby Burns is from Dixie, a bay daughter of the brown Dictator appearing in some of the first tables in this paper. The colors of all foals found from Dispute and Bobby Burns are:

Dispute: bay, 3; black, 2; gray, 4; all, 9.

Bobby Burns: bay, 40; black, 10; brown, 10; chestnut, 3; gray, 43; all, 106.

This is certainly a different kind of gray from the others just described, but I have not enough data to try to explain it.[1]

[1]In connection with the bay and gray factors I may quote the following from the pen of Professor Karl Pearson ("The Law of Ancestral Heredity," *Biometrika*, 1903, vol. 2, p. 214), though it was written about the English thoroughbred: "If black or gray coat-colour in horses were 'recessive,' when two blacks were mated we should expect only black offspring, but black can disappear for a generation or even two and then reappear. Or, take a case like that of a gray horse Viscount,

The last character I have to deal with is roan. This, like gray, is epistatic to the four usual colors in most families, but may not be in all.

Many of the roans of to-day go back to the old roan race-mare Lady Franklin, through her daughter Lady Frank and grandson Jay Bird, both roans. Jay Bird sired Eagle Bird, Jay Hawker, Allerton and Jackdaw, and Jay Hawker sired Jay McGregor. The following table shows all foals but chestnuts.

Stallion.	Color.	Foals.					
		Bays.	Blacks.	Browns	Grays.	Roans.	All not Roan.
Eagle Bird	roan	15	1	4	0	8	20
Jay Bird	roan	54	0	15	3	63	72
Jay Hawker	roan	6	0	0	0	13	6
Total		75	1	19	3	84	98
Jay McGregor	bay	11	1	5	0	0	17
Allerton	brown	32	8	20	2	1	62
Jackdaw	brown	9	3	12	1	0	25

Allerton's roan foal is from a roan daughter of Jay Bird.

Another family goes back to Laura Fair, roan, through her roan granddaughter Spanish Maiden. This last mare produced 1 bay and 3 roans, including the sire Margrave. Tom Hal, Jr., founded another family of roans, and another goes to the roan mare Tilla, which had 4 bay foals, 1 brown and 3 roans, the latter including Fred S. Wilkes. The Brown Hal appearing in the table below is a son of Tom Hal, Jr. Chestnut foals are omitted as before.

Stallion.	Color.	Foals.	
		Not Chestnut or Roan.	Roan.
Fred S. Wilkes	roan	13	8
Margrave	roan	35	30
Tom Hal, Jr.	roan	7	7
Total		55	45
Brown Hal	brown	61	4

where gray remained dominant for three generations only to disappear before the chestnut of the mare Blue Stocking in the Viscount and Blue Stocking filly Miss Johanna!" Just what that passage was intended to mean is a problem which I have not yet solved. What do blacks produce when mated together, and what has that to do with skipping a generation or so? And if a recessive cannot skip what can? Certainly not a dominant. What is to prevent us from supposing Viscount a heterozygote?

The roan and also the two gray foals of Brown Hal probably get their color from their dams, since in all cases these were daughters of roan or gray sires. As to the kind of roans these are I can only say that Margrave and one of his foals, and one of the foals of Tom Hal, Jr., are all red roans.

I have found six roan foals which had neither parent roan. One was from a dun mare. One was from two bays and another was from a chestnut and a bay, the chestnut being Robert McGregor, which almost certainly carried no gray factor. Another was from a bay sire and a chestnut dam. The other two each had a gray parent, and at least one of them was a black roan. Now only six roans produced by the thousands of cases of mating together horses not roan is a very small percentage. It is to be noticed that these six are not closely related. Even the two grays concerned are one Pilot, Jr., and one Sontag Mohawk. It seems to me probable that red roan at least is an ordinary dominant, and that all but one of the above cases are mistakes or exceptions. It is quite possible that all horses having the factor *R* are roan, the type of roan depending upon the color the horse would have been if he had not had that factor. This, if correct, explains away the difficulty presented in the next paragraph in so far as roan is concerned.

The relation between gray and roan is not clear. It seems probable that some of the *black* roans may be connected with gray, but an examination of the tables given above will convince one that, in general, the two colors are quite distinct. I have found only two instances of the mating together of grays and roans, and in both the result was gray. Of course much more evidence would be needed in order to find out how they act toward each other. The relation between these two factors and the bay factor is also not quite clear. It is evident that the presence of either can conceal bay, but whether or not either can appear in the absence of the bay factor is not certain. I am inclined to think that they can. If gray cannot we have an explanation of the gray foals from the black Dispute, but get no help in the much harder problem concerning his bay half-brother, Bobby Burns. One would expect to find some chestnuts carrying gray or roan factors (if the *ChhR* horses are chest-

nut roans there should be no chestnuts carrying the roan factor). With one possible exception in the case of each color I have found no such case. How unsatisfactory these two cases are will appear from the color pedigrees of the horses concerned.

Molly Morton, gray.	Banker Rothschild, brown.	Rothschild.
		Pilot Anna, gray.
	Lady Forrester, chestnut.	Royal George, chestnut.
		Belle of Saratoga, brown.
North Wind, roan.	Young Clay Pilot, bay.	Clay Pilot, bay.
		?
	Lola M., chestnut.	Roy Executor, brown.
		Light Dale, chestnut.

SUMMARY.

This study of the pedigrees of blooded trotters indicates that the color of such horses is usually controlled by five factors, as follows. First, a factor for chestnut, *C*, present in all the horses studied. Second, a factor for black, Hurst's factor, *H*, epistatic to the factor *C*, and hypostatic to the three following. Third, a factor for bay, *B*. Fourth, a factor for roan, *R*. Fifth, a factor for gray, *G*. *R* or *G* inhibits *B* if it is present, but whether they depend upon its presence for their own appearance or not is not clear.

COLUMBIA UNIVERSITY,
June, 1910.

THE MARSUPIUM OF THE ANODONTINÆ.

In the June number of the BIOLOGICAL BULLETIN (p. 31), G. Lefevre and W. C. Curtis have published a paper on "the Marsupium of the Unionidæ," in which they say that the lateral (secondary) water tubes cut off from the original (primary) water tubes in the marsupium of the Anodontinæ, described by myself in *Nautilus*, February, 1910, are *not present.* In order to show this, they publish three figures of horizontal sections through the marsupia of three species of Anodontinæ.

To obviate misunderstanding, I want to point out, that, in two of the figures referred to, these secondary water tubes ARE PRESENT, most *beautifully* and *typically* in Fig. 1. In Fig. 3 traces of them are observable, while in Fig. 2 they are *not yet* developed. These tubes are *not* blood vessels, as might be believed after superficial investigation.

For the rest, I cannot go into detail, but must refer to my future publication (illustrated by microphotographs) in the *Memoirs of the Carnegie Museum*, where additional facts will be published.

A. E. ORTMANN.

CARNEGIE MUSEUM, PITTSBURGH, PA.,
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